EXPRESS MAIL NUMBER. EV325967067US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Niboyuki Itoh and Michael Kavanaugh
Application No.	:	09/801,968
Filed	:	March 7, 2001
For	:	HUMAN FGF-23 GENE AND GENE EXPRESSION PRODUCTS

#17
M.Q.
6/11/03

Examiner	:	Christine Saoud
Art Unit	:	1647
Docket No.	:	60219-4 / PP-17150.001
Date	:	June 3, 2003

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AFFIDAVIT OF DR. MICHAEL KAVANAUGH UNDER 37 C.F.R. § 1.132

Sir:

I, Michael Kavanaugh, M.D., being duly sworn, say:

1. I am an internationally recognized scientist and am presently employed as Senior Director, Research, at Chiron Corporation, Emeryville, California (employed at Chiron Corporation from 1994 to present). I received a Bachelors Degree in Molecular Biophysics and Biochemistry from Yale University in 1978 and a M.D. degree from Vanderbilt Medical School in 1983.

2. I am an author or co-author of more than 20 peer-reviewed research articles and have been invited to give numerous presentations on my research at national and international meetings. Prior to joining Chiron Corporation I practiced medicine at the University of California, where I maintain appointments as Associate Clinical Professor of Medicine, and Attending Physician, VAMC, Intensive Care Unit, San Francisco. My curriculum vitae is attached as Exhibit 1.

3. In my capacity as Senior Director, Research, at Chiron Corporation, and in my prior work, I am familiar with identifying and characterizing proteins, and mRNA expression, using methods well-known to those of ordinary skill in the art at the time of filing of the above-identified patent application. I am also familiar with methods of protein expression and methods of assaying biological activity of proteins, particularly growth factors.

4. On information and belief, claims of the present application have been rejected under 35 U.S.C. § 112, first paragraph, and under 35 U.S.C. § 101.

5. It is my opinion as an expert in this art that FGF-23 as claimed has a substantial, credible and real world utility, and based on this, a person of ordinary skill in this area would know how to use the claimed invention.

6. Evidence disclosed in the patent application and herein supports a utility of FGF-23 as a modulator of phosphate levels in the blood. Because of this role, FGF-23 is implicated in treatment of hyperphosphatemia, in cancer-related bone loss, and other pathological and physiological conditions related to bone density and to phosphate levels in the blood. A general outline of bone homeostasis in the human body is shown in Exhibit 2. Normally, release of phosphate from the bone ("resorption") is balanced with addition of phosphate to the bone, during bone formation. In the course of these normal processes, phosphate is transferred to and from the extracellular fluid (ECF). During many disease states, this homeostasis is disrupted. If the resorption step is out of balance with the bone formation, then there is a net loss of phosphate from the bone to the ECF, with excretion of the phosphate from the body. Loss of phosphate from the bone is clinically detected as osteoporosis, rickets, tumor-related bone loss, and numerous other serious pathological conditions, as discussed further below. The results described herein, and initially discussed in the patent application as filed, indicate that the FGF-23 of this invention plays a crucial role in phosphate metabolism, and a mutated form thereof, also disclosed in the application, can contribute to lower phosphate levels in the circulation.

7. The patent application discloses FGF-23 as a new member of the family of growth factors referred to as fibroblast growth factors, FGF. In our initial experiments on FGF-

23 expression, in which FGF-23 was expressed as a C-terminal His-tagged product in a baculovirus/Sf9 system, we noted that two different products could be produced. Both products were secreted into the medium. One product was a ~28 kDa His-tagged polypeptide. A second product was a ~20kDa non-tagged polypeptide. Along with the ~20 kDa polypeptide, a ~7-12 kDa His-tagged polypeptide was also expressed. These results suggested the existence of a cleavage site in the FGF-23 protein closer to the C-terminal end than the N-terminal end.

8. I directed the further analysis of the baculovirus-expressed His-tagged hFGF-23 secreted by Sf9 cells, which revealed a cleavage site between amino acids R179 and S180. This site is distinct from the signal peptide removal site (between P26 and N27) and from an alternate signal peptide cleavage site (between G33 and S34). Additional details of these results are disclosed in the patent application as filed, in Example 2 and Table I at pages 39 and 40.

9. Subsequent to our discovery of this cleavage site, it was demonstrated that mutations at this cleavage site are associated with an inherited disease in humans, resulting in hypophosphaetemia. (The ADHR Consortium, *Nature* 26:345, 2000.) This publication discloses the genetic analysis of four unrelated families afflicted with autosomal dominant hypophosphatemic rickets (ADHR). Three missense mutations were found in these families, in which the missense mutations affected two arginine residues that are three amino acids apart in the FGF-23 gene. Although the authors speculated that these mutations may give rise to ADHR, the publication contained no data regarding a role of FGF-23 protein, or a mutant thereof, in regulating circulatory phosphate levels. ADHR is a member of the group of diseases related to disturbances in phosphate metabolism, which are generally characterized by low serum phosphorus concentrations, rickets, osteomalcia, lower extremity deformities, short stature, bone pain, and dental abscesses. In view of the serious effects of these diseases, and the inherited nature, it is of great scientific interest and medical importance to identify the underlying mechanisms of the disturbances in the phosphate metabolism.

10. Bowe *et al, B.B.R.C.* 284:977 (2001) subsequently showed that FGF-23 expression is elevated in tumor-induced osteomalcia (TIO). Osteomalcia refers to a condition in which bones become softened, which leads to brittleness and breakage. Thus, in addition to

inherited conditions described in paragraph 9 above, disturbances in phosphate regulation are also implicated as contributory factors in the deteriorating health of patients suffering from cancer.

11. Experiments performed in my laboratory and under my supervision have identified a role of non-cleavable FGF-23 (in which arginine at position 179 is substituted with glutamine) in regulating serum phosphate levels in mice, and have further shown the utility of FGF-23 in treating disease states related to disturbances in phosphate metabolism. Such a mechanism was disclosed in the patent application at, for example, page 8, lines 1-7. These experiments were performed as described below:

12. FGF-23 (R179Q) protein was expressed in baculovirus, purified by 6XHis affinity chromatography, dialyzed against phosphate-buffered saline (PBS), and filter sterilized, essentially as described in Example 2 of the patent application. 6-week-old mice of the Balb/c strain were used for these experiments. FGF-23 (R179Q) protein (160 μl, or about 4 μg) or a PBS control (160 μl), was injected intraperitoneally (IP) three times, at intervals of 5 hours. Blood was collected by cardiac puncture and the animals were sacrificed at 24 hours (i.e. 14 hours after the last injection). The serum phosphate, calcium, potassium and sodium levels were measured, using standard methods known in the art, and the results are shown in Table 1:

Table 1. Systemically Administered Non-cleavable FGF-23 (R179Q) Acutely and Specifically Lowers Serum Phosphate Levels in Mice

Treatment group	PBS control	FGF-23 R179Q
Serum phosphate, mg/dL	9.2 ± 0.8	5.9 ± 0.8 [1]
Serum calcium, mg/dL	9.4 ± 0.4	9.4 ± 0.4
Potassium, mg/dL	4.0 ± 0.4	4.4 ± 0.5
Sodium, mg/dL	150 ± 0.2	148 ± 0.2

[1] $p < 0.0001$ vs. PBS control

13. As shown above in Table 1, systemically administered non-cleavable FGF-23 (R179Q) acutely and specifically lowered serum phosphate levels in mice from a control (PBS treatment) level of 9.2 ± 0.8 mg/dL to a treatment level of 5.9 ± 0.8 mg/dL. In contrast, no significant effect was seen on the serum calcium, potassium, or sodium levels.

14. We also investigated the time course of the effect of FGF-23 R179Q on serum phosphate levels. This experiment was performed as described below:

15. FGF-23 (R179Q) protein was expressed in baculovirus, purified by 6XHis affinity chromatography, dialyzed against phosphate-buffered saline (PBS), and filter sterilized, essentially as described in Example 2 of the patent application. 6-week-old mice of the Balb/c strain were also used for these experiments. FGF-23 (R179Q) protein (160 μl, or about 4 μg), or a PBS control (160 μl), was injected intraperitoneally (IP) three times, at intervals of 5 hours. Blood was collected by cardiac puncture and the animals were sacrificed at 24 hours (i.e. 14 hours after the last injection), at 48 hours, and at 72 hours. The serum phosphate, calcium, potassium, and sodium levels were measured, using standard methods known in the art, and the

results are shown in Table 2. As in the experiments described in paragraphs 12 and 13 above, FGF-23-treated mice showed an acute, statistically significant reduction in serum phosphate levels at 24 hours, from a control level of 7.2 mg/dL to a treatment level of 5.4 ± 0.2 mg/dL . This effect was no longer seen at 48 hours and 72 hours. In these time-course experiments, no effect on serum calcium potassium, or sodium was detected.

Table 2. Effect of FGF-23 (R179Q) on Serum Phosphate Levels in Mice: Time Course

Treatment group	**PBS control**	**FGF-23 R179Q**
Serum phosphate, mg/dL, 24 hours	7.2	5.4 ± 0.2 ($p<0.01$)
Serum phosphate, mg/dL, 48 hours	7.4 ± 1.0	10.2 ± 1.2 (p=NS)
Serum phosphate, mg/dL, 72 hours	8.2 ± 0.4	7.4 ± 1.4 (p=NS)

16. Based on these results and the information disclosed in the patent application, it is my opinion, as an expert in this art and as a physician knowledgeable in the causes and treatment of bone-related diseases, that FGF-23 plays a significant and specific role in regulating serum phosphate levels. Hyperphosphatemia can also be caused by acute and chronic renal insufficiency, hypoparathyroidism, pseudohypoparathyroidism, abnormal circulating parathyroid hormone, acromegaly, tumoral calcinosis, and administration of bisphosphonates. Hyperphosphatemia can reflect increased entrance of phosphate into the ECF, as a result of neoplastic diseases such as leukemia and lymphoma; increased catabolism; and respiratory acidosis. It is my opinion that the FGF-23 and FGF-23 R179Q of the invention clearly have utility in a variety of disease states wherein the circulating phosphate levels in the blood indicates the need for treatment.

17. I further declare that all statements made herein of my own knowledge are true and that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code.

W. Michael Kavanaugh

State of California)
) ss.
County of Alameda

On this 29 day of May, 2003, before me, a Notary Public in and for the State and County aforesaid, personally appeared W. Michael Kavanaugh, to me known and known to me to be the person of that name, who signed and sealed the foregoing instrument, and he acknowledged the same to be his free act and deed.

Notary Public

Commission expires May 10, 2004

ELISSA M. NASH
Commission # 1262307
Notary Public - California
Alameda County
My Comm. Expires May 10, 2004

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William Michael Kavanaugh, M.D.

Curriculum vitae

PERSONAL DATA

Maritus Status: Married, 1 child Citizenship: USA Residence: Orinda, CA

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EDUCATION

Undergraduate: Yale University, B.A., Molecular Biophysics and Biochemistry, 1978
Medical School: Vanderbilt Medical School, M.D., 1983

EMPLOYMENT AND EXPERIENCE

Academic/Clinical

7/83 - 6/84	Internship, Internal Medicine, University of California, San Francisco
7/84 - 6/86	Residency, Internal Medicine, University of California, San Francisco
7/86 - 6/88	Research Fellow, Cardiovascular Research Institute , L.T. Williams laboratory, University of California, San Francisco
7/88 - 12/89	Clinical Fellow in Cardiology, University of California, San Francisco
3/90 - 6/92	Instructor in Medicine, University of California, San Francisco
7/92 - 6/95	Assistant Adjunct Professor of Medicine, UCSF
8/96 – 7/01	Assistant Clinical Professor of Medicine, UCSF
7/01-present	Associate Clinical Professor of Medicine, UCSF
1/90 - present	Attending Physician, VAMC, Intensive Care Unit, San Francisco (WOC)

Industry

11/94 - 4/95	Principal Scientist, Chiron Corporation
4/95 - 9/95	Senior Scientist, Chiron Corporation
9/95 - 3/97	Associate Director of Biology Discovery, Chiron Corporation
3/97 - 1/99	Director, Biological Discovery, Chiron Corporation
1/99 - present	Senior Director, Research, Chiron Corporation

HONORS, AWARDS AND LICENSES

5/78	B.A. *magna cum laude*, Molecular Biophysics and Biochemistry, Yale University.
8/81	Alpha Omega Alpha medical honor society
5/83	Graduated first in class, Vanderbilt Medical School -Upjohn Award for Excellence, Vanderbilt Medical School -Founder's Medal, Vanderbilt Medical School
1984	California Medical License (active, #G53774)
7/86	American Heart Association Fellowship Grant
11/86	National Research Service Award (NIH - 2 years)
9/86	Board Certified, Internal Medicine (active, #109439)
9/88	American Federation for Clinical Research
11/89	Board Certified, Cardiology (active, #109439)
3/90	Physician Scientist Award (NIH - 5 years)
9/91	Fellow, Program of Excellence in Molecular Biology
1997-2000	AHA Western States Affiliate Grant Peer Review Committee

RESEARCH INTERESTS AND EXPERIENCE

- Growth Factors, Cytokines, Chemokines, Receptors, Intracellular Signaling, Therapeutic Proteins, Antibodies, Protein Engineering
- Molecular Biology, Cell Biology, Protein Biochemistry, Preclinical Pharmacology
- Cancer Biology And Immunology, Cardiovascular Biology and Physiology, Wound Healing
- Gene Discovery, Target Discovery And Validation, Therapeutic Protein Discovery And Development Including Therapeutic Antibodies, Small Molecule Discovery, Gene Therapy Research
- Preclinical And Clinical Development Through Phase III Planning, Practicing Physician
- Research Management, Project Management, Program Management

- Current Positions: Leader, Therapeutic Antibody Research Program, Chiron; Attending Physician, ICU, San Francisco VA Medical Center; Associate Clinical Professor of Medicine, UCSF

ISSUED PATENTS AND PUBLISHED PATENT APPLICATIONS

US patent #5744313. Assay employing novel protein domain which binds tyrosine phosphorylated proteins. 4/28/98
US patent #5925547. Nucleic acid encoding novel protein domain which binds tyrosine phosphorylated proteins 7/20/1999
US patent #6090621. Signaling inositol polyphosphate 5-phosphatases (SIPs) 07/18/2000
US patent #6280964. Binding sites for phosphotyrosine binding domains 08/28/2001

WO 97/40173 PI 3-kinase fusion mutants and uses thereof
WO 98/00539 Mitogen-activated protein kinase) kinase-3 (mkk3) interacting protein (MIP)
WO 00/18921 Synthetic peptides having FGF receptor affinity
WO 00/21548 Angiogenically effective unit dose of FGF and method of administering

WO 00/46380 Fibroblast [illegible]wth factor receptor-immunoglobulin f[illegible]
WO 00/56890 Human FGF gene and gene expression products
WO 01/13031 Dose of an angiogenic factor and method of administering to improve myocardial blood flow
WO 01/14415 EGFH2 genes and gene products
WO 01/31008 Human FGF-20 gene and gene expression products
WO 01/36640 Human FGF-21 gene and gene expression products
WO 01/66595 Human FGF-23 gene and gene expression products

REPRESENTATIVE PUBLICATIONS

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Kavanaugh WM, Williams LT, Ives HE, and Coughlin SR (1988) Serotonin-Induced Deoxynucleic Acid Synthesis in Vascular Smooth Muscle Cells Involves a Novel, Pertussis Toxin-Sensitive Pathway. *Molecular Endocrinology* **123**: 599-605.

Kavanaugh WM, Harsh IV GR, Starksen NF, Rocco CM, and Williams LT (1988) Transcriptional Regulation of the A and B Chain Genes of Platelet-Derived Growth Factor in Microvascular Endothelial Cells. *J. Biol. Chem.* **263**: 8470-8472.

Harsh IV GR, **Kavanaugh WM**, Starksen NF and Williams LT (1989) Cyclic AMP Blocks Expression of the c-*sis* Gene in Tumor Cells. *Oncogene Research* 4: 65-73.

Escobedo, JA, Kaplan, DR, **Kavanaugh, WM**, Turck, CW and Williams, LT (1991) A Phosphatidylinositol-3 Kinase Binds to Platelet-Derived Growth Factor Receptors Through a Specific Receptor Sequence Containing Phosphotyrosine. *Mole. Cell. Biol.* 11:1125-1132.

Escobedo, JA, Navankasattusas, S, **Kavanaugh, WM**, Milfay, D, Fried, VA and Williams, LT (1991) cDNA Cloning of a Novel 85 Kd Protein That Has SH2 Domains and Regulates Binding of PI3- Kinase to the PDGF ß-Receptor. *Cell* **65**:75-82.

Turck, CW, Escobedo, JA, **Kavanaugh, WM**, and Williams, LT. (1991) Structural and Functional Characterization of a Synthetic Phosphorylated Peptide Derived from the PDGF ß-Receptor. *Pept. Res.* 4: 36-39.

Kavanaugh, WM, Klippel, A, Escobedo, JA and Williams, LT. (1992) Modification of the 85 kD Subunit of Phosphatidylinositol 3' Kinase in Platelet-Derived Growth Factor-Stimulated Cells *Mole. Cell. Biol.* **12**: 3415-3424.

Kavanaugh, WM. Platelet-Derived Growth Factor: Future Directions in the Prevention of Restenosis. (1993) In:Interventional Cardiology:Future Directions, 2nd Edition, John HK Vogel, and Spencer B. King, Eds. Mosby-Yearbook, Littleton, MA

Kavanaugh, WM, Turck, CW, Klippel, A and Williams, LT. (1994) Tyrosine 508 of the 85 kDa Subunit of Phosphatidylinositol 3-Kinase is Phosphorylated by the Platelet-Derived Growth Factor Receptor. *Biochemistry* **33** (36): 11046-11050.

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Vollenweider, P, Clodi, M, Martin, SS, Imamura, T, **Kavanaugh, WM**, and Olefsky, JM. (1999) A SH2 domain-containing 5' Inositolphosphatase Inhibits Insulin-induced GLUT4 Translocation and Growth Factor-induced Actin Filament Rearrangement. *Mole. Cell. Biol.*,**19(2)**:1081-91.

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Abraham, JA, Yeng, S, Terjung, R and **Kavanaugh, WM**. (manuscript in preparation). Therapeutic Benefit of Intramuscular Delivery of FGF-2 in a Rat Model of Peripheral Vascular Disease.

Abraham, JA, Yeng, S, Terjung, R and **Kavanaugh, WM** (manuscript in preparation). Prolongation of the Therapeutic Effect of Intramuscular FGF-2 by Repeat Dosing in a Rat Model of Peripheral Vascular Disease.